Message

From: Huang, Hwa [huang.hwa@epa.gov]
Sent: 11/18/2019 4:47:01 PM
To: Hill, Donna [Hill.Donna@epa.gov]; Johnsie Lang [jrlang@ncsu.edu]; Chernoff, Neil [Chernoff.Neil@epa.gov]; Strynar, Mark [Strynar.Mark@epa.gov]; Lindstrom, Andrew [Lindstrom.Andrew@epa.gov]
Subject: RE: Decision on Manuscript ID ez-2019-006809

This is the paragraph in the paper concerning how the dosing solution was made:

Ex. 5 Deliberative Process (DP)

Hwa

From: Hill, Donna <Hill.Donna@epa.gov>
Sent: Monday, November 18, 2019 11:39 AM
To: Johnsie Lang <jrlang@ncsu.edu>; Chernoff, Neil <Chernoff.Neil@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; Huang, Hwa <huang.hwa@epa.gov>
Subject: RE: Decision on Manuscript ID ez-2019-006809

The control dosing solution was based on the NBP2 dosing solution's strongest concentration of ethanol. (the highest-dosed males) It did contain ethanol.

I am surprised and angered at the strong language used by the editor who is ready to dismiss the paper.

Donna

From: Johnsie Lang <jrlang@ncsu.edu>
Sent: Monday, November 18, 2019 10:42 AM
To: Chernoff, Neil <Chernoff.Neil@epa.gov>; Hill, Donna <Hill.Donna@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; Huang, Hwa <huang.hwa@epa.gov>
Subject: Fwd: Decision on Manuscript ID ez-2019-006809

---------- Forwarded message ---------
From: **Environmental Science & Technology Letters** <onbehalfof@manuscriptcentral.com>
Date: Mon, Nov 18, 2019 at 9:35 AM

Subject: Decision on Manuscript ID ez-2019-006809
To: <jrlang@ncsu.edu>

18-Nov-2019

Journal: Environmental Science & Technology Letters
Manuscript ID: ez-2019-006809
Title: "Toxicity of Balb-C Mice Exposed to 1,1,2,2-Tetrafluoro-2-[1,1,1,2,3,3-hexafluoro-3-(1,1,2,2-tetrafluoroethoxy)propan-2-yl]oxyethane-1-sulfonic acid (PFESA-BP2)"
Author(s): Lang, Johnsie; Strynar, Mark; Lindstrom, Andrew; Farthing, Amy; Huang, Hwa; Schmid, Judith; Hill, Donna; Chernoff, Neil

Dear Dr. Lang:

Thank you for submitting your manuscript for publication in Environmental Science & Technology Letters. The manuscript was forwarded to several reviewers for their consideration, and the reviews are enclosed. After careful consideration, I regret to inform you that the manuscript cannot be considered further for publication in Environmental Science & Technology Letters.

The reviewers have expressed serious reservations regarding the suitability of your manuscript for publication which cannot be addressed without major revision. Of primary concern is the lack of a suitable control for the carrier used for the toxicant.

Given the comments of the reviewers, you will need to consider submission of this work elsewhere.

I hope that the reviewers' comments are of help to you if you choose to revise the manuscript for submission to another journal.

Sincerely,

Dr. Daniel Schlenk
Associate Editor
Environmental Science & Technology Letters
Email: schlenk-office@estlett.acs.org
Phone: 951-827-2018
Fax: 202-354-4613

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments:
This paper reported the toxic effects of PFESA-BP2 on mice. In this study, mice were dosed with PFESA-BP2 at 0, 0.04, 0.4, 3, and 6 mg/kg-day for 7 days by oral gavage. Authors found that treatment with PFESA-BP2 at 3 and 6 mg/kg-day caused liver injury. Authors also found higher accumulations of PFESA-BP2 in the liver compared to serum. Although this study is novel, I think there are some serious flaws in this study design, which make it difficult to obtain convincing experimental data.

1. In this study, PFESA-BP2 was dissolved in ethanol followed by dilution with deionized water for a final concentration of 1 g/L in 90:10 H2O: ethanol. In this stock solution, the volume concentration of ethanol is 10%, and its mass concentration is 78.9 g/L. That is, the concentration of ethanol in the stock solution is 78.9 times that of PFESA-BP2. Therefore, no matter how diluted PFESA-BP2 is administered to mice, it means that the mice are simultaneously exposed to ethanol at concentration of 78.9 times of PFESA-BP2. According to this calculation, mice were administered with 6 mg/kg of PFESA-BP2 per day, and were simultaneously exposed to 473.4 mg/kg of ethanol. It is equivalent to a person with 60 kg body weight drinking more than 56 ml liquor with 50% alcohol content per day! It is well known that ethanol has strong hepatotoxicity. How did authors know the liver injury in the exposed mice is caused by PFESA-BP2 instead of ethanol?
2. Did the authors use mice that are gavaged with deionized water as a controls? This paper did not indicate whether the control mice were orally administered with a solution containing the same concentration of ethanol. However, since the ratio of ethanol concentration to PFESA-BP2 concentration in the stock solution is fixed, the exposure dose of ethanol in each group of mice is also changed by the diluted solution, so each exposed group needs a corresponding control. Obviously, the authors did not set a control group with the same exposure dose of ethanol for each exposure group.

Based on the above points, I believe the liver injury in the exposed mice in this study is more likely caused by ethanol rather than PFESA-BP2. Therefore, I think this article is not suitable for publication in EST Letter.

Reviewer: 2

Comments:
This is an important paper that provides the first toxicology information on PFESA-BP2, a newly identified PFAS that was found in the blood serum of North Carolina residents as a result of industrial contamination of their drinking water source, the Cape Fear River. It is notable that this compound appears to cause liver toxicity and bioaccumulate in humans and mice similarly to more thoroughly studied long-chain perfluoroalkyl acids such as PFOA and PFOS.

My specific comments are:
Line 1 (title) and Lines 20-21 (abstract). Suggest mentioning in the title and abstract that the compound studied is a newly identified PFAS.

Lines 43-51. Suggest specifically mentioning that, like PFOA and PFOS, PFESA-BP2 is an 8 carbon PFAS when describing it and comparing its structure to PFOS.

Line 75. Can the average or median concentration of PFESA-BP2 in the serum samples be provided?

Lines 74-81. Can this discussion be expanded to say that this is the first (or one of the first) demonstrations that PFAS other than perfluoroalkyls, such as PFESA-BP2 with oxygens in the chain, bioaccumulate in humans? And that this may be related to longer the chain length of PFESA-BP2, since the 6 carbon perfluoroether HFPO-DA did not appear to bioaccumulate from the Cape Fear River drinking water source to the residents' blood serum?

Lines 195-198. Please further clarify the dose groups in which these effects occurred. Were the livers enlarged and pale in all dose groups of treated mice? Was there 1 reticulated liver in both male and female control groups and in each of the male and female lower dose groups?

Line 203. Should this be changed to "Livers from the 0.04 and 6 mg/mg/kg-day dose group were not analyzed with histopathology"?

Lines 199-212. From the information in the Methods section, histopathology was performed on only 1 control, 0.4 mg/kg/day, and 3 mg/kg/day liver of each sex. Are these small numbers sufficient to conclude that these changes do not occur in the controls? Suggest mentioning small numbers for all of the effects, not just cell death as mentioned in

the last sentence.

Lines 225-227. Please state the average serum concentrations in Wilmington, NC residents.

Lines 229-230. "The concentrations of PFESA-BP2 are in the range of previously reported mouse serum PFOA/PFOS concentrations." Are these comparisons based on the same doses/exposure durations of PFESA-BP2 and PFOA/PFOS? Please provide more details as to the basis of this comparison.

Lines 238-243. Could the detections in control livers be due to inadvertent exposure to PFESA-BP2. Is it possible that it was not detected in the serum of these animals because it bioaccumulates to a greater extent in liver than in serum (i.e. liver levels from inadvertent low exposures would be higher than serum levels)?

Lines 256-257. Future work. In the Methods section, it is stated that a portion of the liver was stored for future PCR analysis. Would this analysis provide information on the potential mode of action of PFESA-BP2?

Line 276. Table 2. Footnote b is not defined.

Line 284. Citation 3. It appears that something is missing from the beginning of this citations.

Figure S1. Please add information about what the slides in the left versus right columns show (e.g. different magnifications?).

Table S1. Suggest rewording the title to "Number of animals per dose group for parameters evaluated" or similar.

Table S1. The units of dose (mg/kg-day) should be stated

Table S1. It is unclear why the 2nd dose group for females is labeled "0.3, 0.35, 0.4). Unless I missed it, this range of doses was not mentioned in the paper.
